A TINY PROTEIN BORROWED FROM ANCIENT CREATURES HAS THE POWER TO TRANSFORM MEDICINE—IF WE CAN JUST FIGURE OUT THE BEST WAYS TO WIELD IT | BY JASON BITTEL

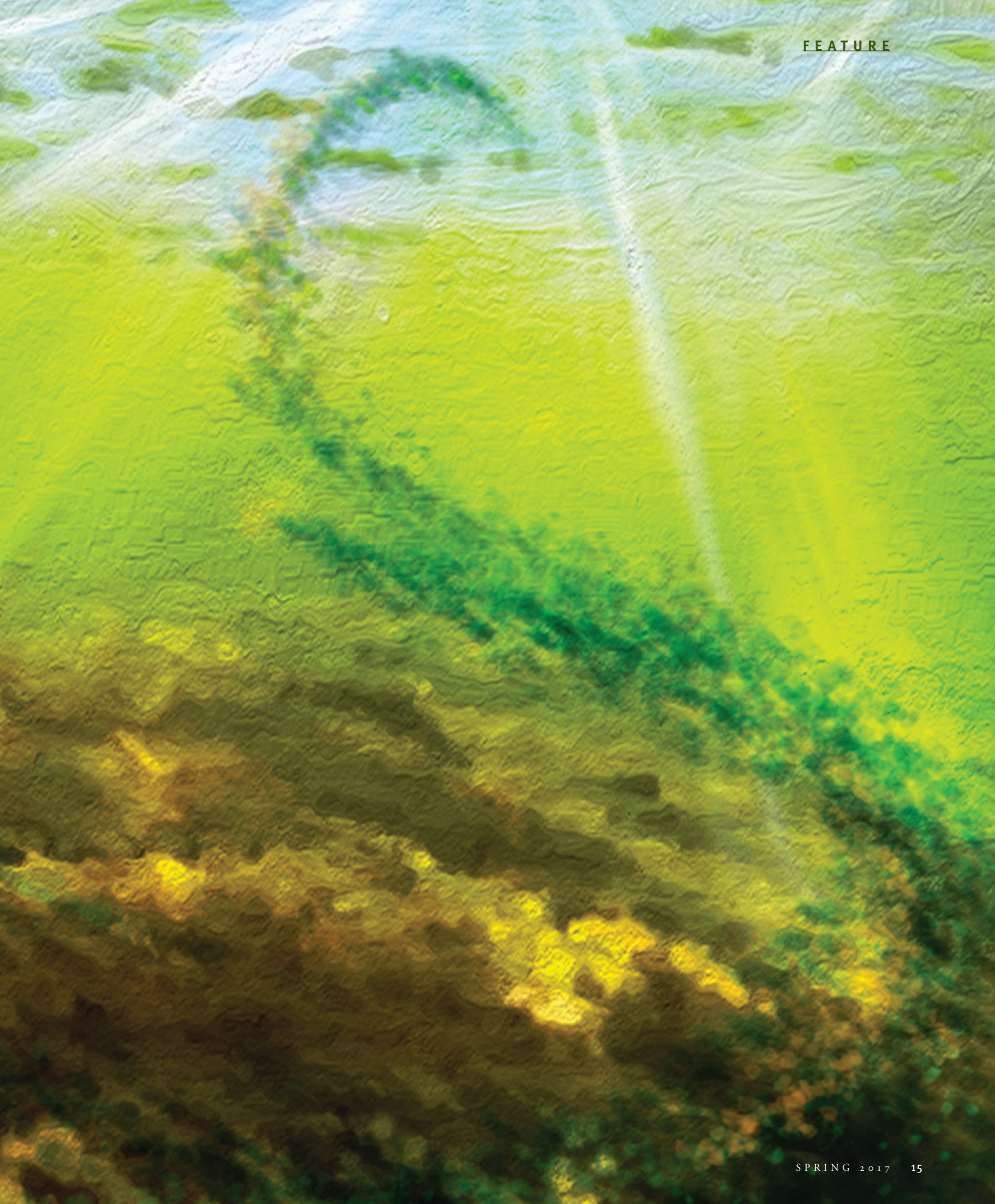
LET THERE BE LIGHT

It's daybreak in the swamp. As the sun peeks over the horizon, its rays crawl across the water and begin to penetrate the murky depths. Below, millions of single-celled organisms sense the light and start flapping their flagella in a desperate attempt to chase down the beams. These life forms eat sunlight by way of photosynthesis, and each day they must migrate out of the dark to harvest photons.

Why exactly are we talking about green algae and their daily sun salutations? Well, only because algae innards have yielded one of the greatest scientific tools of the modern era, and it seems fitting that we should give credit where it's due.

Most scientists believe single-celled aquatic plants like these emerged as many as 1.5 billion years ago. They are among the most primeval life forms. But in just the past decade—an infinitesimally small blink in the history of Earth—we humans have found a way to steal a piece of these creatures and inject it into mice, monkeys, and even humans. We call the tool optogenetics, and scientists at the University of Pittsburgh are using it to unlock some of biology's greatest secrets—from how the brain and central nervous system function to the circuits that govern mental health issues and chronic pain. Someday soon, optogenetics may even allow us to restore sight to the blind or turn whole organs on and off with little more than the press of a button on a remote control.

ILLUSTRATION | MICHELLE LEVEILLE



In 2007, a Stanford researcher named Karl Deisseroth and his then-PhD student, Feng Zhang, introduced these light-sensitive genes into the motor cortex of a mouse. Then, by surgically inserting a fiber-optic wire into the animal's skull, they found a way to bathe these cells in blue light. Turn the light on, and the animal would scamper around in circles. Turn the light off, and the mouse would immediately stop running laps and go back to whatever it was doing before—sniffing the walls or grooming itself.

As you might imagine, this was a pretty big deal. Not only did the researchers prove you could modify the behavior of an animal simply by flipping the equivalent of a light switch, they showed we could hack into and control *individual* neurons by genetically targeting them.

"I certainly had hope that it would be versatile and widely used," says Stanford's Deisseroth, who received the University of Pittsburgh's Dickson Prize in Medicine in 2015. "The potential was clear, but in science anything and everything can go wrong."

About that… The thing about optogenetics is that it's a simple technology, hewn from a simple organism. Flick a switch and see what happens. But porting that technology into humans is akin to taking the switch out of a toaster oven and using it to pilot a nuclear submarine.

You probably know that the brain is composed of neurons. But what you might not realize is that there are thousands of different kinds of these cells, and they differ in size, shape, and the way they interact with one another. Scientists have been sticking electrodes into the brain to stimulate this or that region since the 1700s, and while these experiments were revealing in their own ways, they lacked the finesse necessary to pick through the intricacies of the most advanced organ evolution ever spawned.

"There are just so many different components in the same area, and there's no way to reliably stimulate one electrically without stimulating everything else that's nearby," says Bryan "Mac" Hooks, an assistant professor of neurobiology at the University of Pittsburgh.

But now, the discovery of optogenetics has provided a workaround. All of a sudden, scientists can selectively activate specific kinds of neurons and see what happens. Conversely, through the addition of other light-sensitive opsins that act as inhibitors, like halorhodopsin and archaerhodopsin, they can silence neurons and record what happens (or doesn't) in their absence.

For Hooks, optogenetics means being able to study different kinds of neurons in the hopes of rooting out what circuits control plasticity. Some victims of stroke, for instance, are less able to recover than others. But if we could identify the neurons in the motor cortex that govern this process, then perhaps we could activate or dampen them as needed to make a patient's brain more willing to relearn how to walk, say.

"It's not too science fiction-y to imagine that different cell types have different gene expression patterns, and the products of some of these genes are a variety of ion channels, receptors, and intracellular signaling molecules," says Hooks. "If you find the drug that manipulates specific receptors or ion channels expressed by that cell type and can deliver it to the right place, then maybe you have an opportunity to help somebody who needs to reactivate plasticity in their brain following some kind of debilitating condition, like a traumatic brain injury."

Sarah Ross, who is also an assistant professor of neurobiology, is using a similar process to study the phenomenon of "wind-up." First described around 50 years ago, wind-up is what happens when pain receptors repeatedly fire messages at the brain to tell it something hurts, triggering bigger and bigger responses as time goes on. (If you're reading this with the fascination that can only come from never having experienced wind-up, then consider yourself lucky.)

Even though wind-up was discovered and studied half a century ago, the neural circuits responsible for the excruciating pain amplification have remained a mystery. But recently, Ross and her lab have been using opsins to investigate the condition.

"We showed, using optogenetics, that if you use inhibitory opsins to inhibit a particular subtype of spinal interneurons, you don't get the wind-up," she says.

Unfortunately, this doesn't mean doctors can flick a switch and turn off wind-up in post-surgery patients just yet. But they're not light-years away, either.

"Pain is a major low-hanging fruit," says Brian Davis, a professor of neurobiology and medicine.

If a particular organ is causing chronic pain, and we can identify the channels it uses to send those messages to the brain, then, Davis says, it should be relatively easy to express an inhibitory opsin in those sensory neurons and toggle them with optogenetics.

"You just have a little light, and when your pain gets bad, you turn on your light and the pain goes away," he says. "Think about that—think about the applications!"

In fact, Davis says we're closer than you might imagine. Hooks, Ross, and countless other researchers use mice that have been genetically engineered to produce opsins in particular cell types. But that's not going to work in humans, says Davis, because we're not transgenic mice. And that's where the viruses come in.

In the 1970s, scientists learned that they could use a virus's natural ability to penetrate cells as a way to smuggle other components inside. This is what's known as a viral vector, and they're already in use in humans. In fact, Davis is collaborating with a professor of microbiology and molecular genetics at Pitt named Joseph Glorioso, who has used these microscopic Russian nesting dolls to insert genes that coax a cancer patient's body to produce more of its own, natural opioids. So far, he has shown in a small clinical trial of 10 patients that the delivery system can reduce pain in people who no longer respond to strong pain relievers, like morphine.

While Glorioso's opioid work doesn't employ optogenetics, the virus he's working with, a defanged version of herpes simplex, is a natural neuron invader. This makes it an excellent candidate for becoming an opsin mule.

Davis, Glorioso, and their partners would also like to be able to control another important function—urination. "Spinal cord injury patients have a real problem with urination," says Davis, "and it's actually their number one complaint"—even over loss of sensation and paralysis itself.

For some patients, the worst part may be the constant need to empty the bladder mechanically or the annoyance of constant leakage. But for others who still retain some level of sensation, it's the extremely painful feeling of having to go but being helpless to do anything about it.

Yet manipulating an entire organ will be more difficult than overriding one pain channel. For starters, bladder release is regulated by two separate sphincters—one controlled by skeletal muscle and another by smooth muscle—as well as a muscular layer on the organ's exterior that aids with contraction. So the ideal application would seamlessly incorporate stimuli across this complex network, but do so in a way that is itself not more cumbersome than the symptoms the patients are already experiencing.

Here's a solution: How about a remotely controlled optogenetic bladder sock?

Don't scoff. Thanks to an aggressive outcomes program called SPARC (Stimulating Peripheral Activity to Relieve Conditions), which is supported by the National Institutes of Health Common Fund, Davis and others are already assembling the pieces. A collaboration involving Glorioso, Davis, Kathryn Albers (in the Department of Medicine), and William Goins (of Microbiology and Molecular Genetics) has already shown in a not-yet-published study that they could express channelrhodopsin in sensory neurons in the bladders of mice and cause a contraction with a blast of blue laser light. Another SPARC fellow, a colleague in St. Louis, has been developing a mesh embedded with micro-LEDs, complete with their own power source and ability to be controlled through telemetry. One of the biggest hurdles seems to be getting the optogenetic proteins to express in the right cells, at high enough levels, to actually trigger organ-wide responses.

Porting that technology into humans is akin to taking the switch out of a toaster oven and using it to pilot a nuclear submarine.

Consider the eyeball. José-Alain Sahel, who leads Pitt's Department of Ophthalmology, is using optogenetics to re-activate cells within the retina that have gone dormant. But it's not enough to simply turn the cells back on. Patients need to be able to translate natural light into the specific wavelengths that their cells have been newly programmed to be receptive to. This requires a special set of glasses equipped with cameras and a projection system.

There's still much about this approach that needs to be worked out, but in mice, the researchers showed that resensitized photoreceptor cells could exhibit a full visual cycle, activate cortical circuits, and contribute to behaviors that require some form of vision.

William Stauffer, assistant professor of neurobiology, has been trying to address some of these same issues posed by optogenetics—only the cells he studies are neurons that govern dopamine release. Among other things, dopamine neurons go crazy any time a creature experiences an unpredicted reward. This is why an unexpected bonus at work is more exciting than your monthly paycheck.

This may seem like a subjective difference—the bonus somehow just feels *better*—yet the brain's reaction is actually quantifiable. In the 1980s and '90s, a neurobiologist named Wolfram Schultz used single neuron recording in nonhuman primates to show that dopamine neurons responded to rewards that were better than predicted, but that these same cells were silent when the rewards were worse than predicted.

What's more, you can define these responses mathematically.

For Stauffer, who completed his postdoc under Schultz, optogenetics promised the ability to probe dopamine networks in new and exciting ways. However, he faced the problem of being able to target dopamine neurons and only dopamine neurons (specificity), as well as still being able to achieve high levels of light activation (sensitivity).

"Sensitivity and specificity are *not* independent," says Stauffer, "and it is often necessary to trade off one to get the other."

Fortunately, Stauffer's team was able to bypass this issue by using separate adeno-associated viruses as vehicles for two different opsins—one that was tasked with seeking out the right neurons and another that was designed to deliver high levels of light-sensitive proteins. The old one-two punch, as it were.

But what's even more important is what Stauffer did with his tag team of viruses: He injected them into a rhesus macaque monkey.

This was the first time cell type–specific optogenetics had been achieved in monkeys, notes Stauffer.

Mice have been the optogenetic gold standard for some time: they are small, quick to mature, and notoriously rapid breeders. And, you can order them genetically modified with the light-sensitive proteins already built in.

But mice aren't nearly a perfect model. For instance, the mouse brain is thought to contain about 70 million neurons. That sounds like a whole lot until you learn that the human brain contains 86 *billion*.

"Primate optogenetics will allow us to link structure to function like never before," Stauffer says.

Andrew Schwartz, a Distinguished Professor of Neurobiology, and colleagues figured out how monkeys, and then paralyzed people, could control a robotic arm with just their thoughts. He wants to improve upon that success with optogenetics.

Right now, we can activate peripheral nerves and the muscles they connect to with electric shock, says Schwartz. But this technique tends to recruit certain kinds of muscle fibers to action before others, a glitch that leads quickly to fatigue and lack of power. With optogenetics though, we could generate a small amount of force for a long period of time without getting fatigued. "That's what happens naturally," he says.

Furthermore, the development of opsins responsive to different wavelengths could open up all kinds of other possibilities.

"Suppose you have a bicep and a tricep," says Schwartz. "Say you put a red opsin in one and a yellow opsin in the other. Both muscle nerves join together and travel up the same nerve trunk, so you could put an array of LEDs at one spot on that nerve and selectively activate individual muscles."

As with other startling advances, optogenetics brings new ethical issues to the table. A researcher at Caltech was able to make a mouse attack a latex glove by flipping an optogenetic switch. This sort of demonstration opens the door to speculative scenarios about human behavior dictated by remote control.

Professional bioethicists, however, say practical ethical considerations are more immediately of concern; they cite the need to keep to a minimum the number of people participating in the very first clinical trials that gauge safety and efficacy.

Doctors would be forever altering the nervous system by implanting LEDs and other foreign material. And any time you talk about fiddling around in the brain, there's potential for incurring unforeseen complications. To quote a 2014 review in the *American Journal of Bioethics Neuroscience*, "Optogenetics interventions raise concerns not only for the person who is consenting, but also for the person he or she might become following the intervention, especially if the person experiences negative self-estrangement or significant disruptive personality changes."

The possible clinical applications of the technology are, of course, exciting. Who wouldn't want to be the first to restore sight, end the anguish of chronic pain, or help someone walk again?

But for Deisseroth, who originally coined the term "optogenetics," the appeal is as fundamental as the life forms that made all of this possible.

"I am most excited about the basic science applications," he says. "Some people are thinking about direct clinical applications, but by far the biggest excitement has always been the basic discoveries."

It's daybreak in the swamp, in other words. And we're just getting started. ■